PERMEATION AND MEMBRANE TRANSPORT IN PARASITISM: STUDIES ON A TAPEWORM-ELASMOBRANCH SYMBIOSIS [1]

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It was shown by Read, Simmons and Rothman (1960) that the amino acids L-valine and L-leucine enter the tapeworm, *Calliobothrium verticillatum,* by a process showing adsorption kinetics. The data obtained ruled out simple diffusion but did not permit a definite conclusion as to whether the permeation is a process of active transport. L-valine and L-leucine were each shown to competitively inhibit the entry of the other into the worm. Several other amino acids were shown to inhibit L-valine and L-leucine permeation, but it was not established that these inhibitions were competitive. The studies to be reported have shown that L-valine is actively transported, although further investigation has revealed that when *Calliobothrium* is treated as a member of a symbiotic relationship, the operation of the amino acid entry systems is indeed complex. Appreciation is expressed to Dr. G. Wertheim who lent technical assistance during part of this study, and to the gentlemen of the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts, who furnished many living dogfish.

Materials and Methods

The methods of collection and handling of *Calliobothrium* from the dogfish, *Mustelus canis,* were similar to those used in a previous study (Read, Simmons and Rothman, 1960). The salt solution used in handling both worms and host tissues had the following composition: NaCl, 250 mM.; KCl, 4.4 mM.; $CaCl_2$, 5.1 mM.; $MgCl_2$, 2.9 mM.; urea 300 mM.; and tris (hydroxymethyl) amino methane-maleate buffer, 10 mM. (pH 7.2). All incubations were carried out in this medium, with appropriate experimental additions, at 10° C. In preparing tissues for experimental incubation, the worms were washed in several changes of the salt solution and incubated for 60 minutes at 10° C. before an experiment. Host tissues were removed in ice-cold salt solution and, before the experiment, were incubated at 10° C. for 30 minutes in a large volume of salt solution containing 10 mM. glucose. Throughout this period, the tissue was vigorously aerated with a sintered bubbler attached to a small air pump. For experimental incuba-

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tion, the worms or host tissues were lightly blotted on hard filter paper and transferred to the experimental medium. At the end of an experimental incubation, the worms or host tissues were rinsed by dipping twice in large volumes of the salt solution, blotted quickly on hard filter paper, and placed in a measured volume of 70% ethyl alcohol. It was previously shown that, with occasional shaking, free amino acids are extracted from worm tissues in 50 to 70 per cent alcohol in less than 24 hours (Read, Simmons and Rothman, 1960). In most cases extraction was carried out for over 48 hours but in no case for less than 24 hours. Actually, the concentration in the fluid of the tissues probably comes to equilibrium with the alcohol external to the tissues. The quantity of worm tissue with respect to the volume of the extracting fluid was kept sufficiently low so that an error of less than 1% was introduced by the addition of the worm volume to that of the extracting fluid. Aliquots of alcoholic extracts were used for determination of radioactivity or analysis of amino acids.

Many of the data are expressed in terms of the alcohol-extracted dry weight of tissue; this was determined by heating the extracted tissue for 5 to 6 hours at 100° C. in tared foil pans. Drying for longer periods produced no significant change in dry weight values. Determinations of the alcohol-extracted dry weight/wet weight for 16 worms at the end of a 60-minute incubation in the salt solution at 10° C. gave a value of 0.302 ± 0.016. Nineteen dogfish gut samples, identical with those used in experiments, gave an alcohol-extracted dry weight/wet weight value of 0.135 ± 0.011 after a 30-minute, aerated incubation in salt solution with glucose at 10° C. Plating of extract samples and determinations of radioactivity were carried out as previously described (Read, Simmons and Rothman, 1960).

Two-dimensional chromatography was carried out by a modification of the method of Levy and Chung (1953), as described by Campbell (1960). One-dimensional chromatograms were prepared on Whatman No. 52 paper using sec-butyl alcohol, formic acid, and water (75 : 15 : 10) as the solvent system. Amino acids were quantitatively estimated using the methods of Fowden (1951). Radioautographs of chromatograms were prepared by exposing Eastman "no-screen" x-ray film to the chromatograms after removing the solvent. Histidine was determined by the method of Macpherson (1946). Nitrogen was determined by the micro-Kjeldahl method described by Lang (1958). Other details of methods will be described in context.

Experimental

Further characterization of the amino acid entry systems of Calliobothrium

Several amino acids have been shown to inhibit the penetration of L-valine and L-leucine into *Calliobothrium* (Read, Simmons and Rothman, 1960). In the present study a number of experiments were carried out to determine whether these inhibitions are competitive in nature, and whether there is a reciprocal inhibitory effect of L-valine on penetration of certain of the inhibitory amino acids. An analysis of the inhibitory effects of L-serine, L-threonine, and L-alanine on valine entry showed that the inhibitions indeed are competitive in nature (Table I). Conversely, experimental analysis of the effect of L-valine on the

TABLE I

Effect of L-serine, L-threonine, and L-alanine on the entry of L-valine into Calliobothrium. *S = concentration of L-valine; V = counts per minute per gram of alcohol-extracted dry tissue; N = number of samples*

Amino acid	Inhibitor	N	I/S	V
L-valine-C14	$5 \times 10^{-3}M$ L-serine	4	200	59,575 ± 3673
		4	500	36,200 ± 2876
		4	1000	18,212 ± 1004
		4	2000	11,515 ± 1529
	$2 \times 10^{-3}M$ L-serine	4	200	96,900 ± 4300
		4	500	41,850 ± 1947
		4	1000	24,300 ± 571
		4	2000	16,812 ± 857
	None	4	200	116,600 ± 10,148
		4	500	61,222 ± 5793
		4	1000	46,700 ± 2200
		4	2000	31,775 ± 1520
L-valine-C14	$5 \times 10^{-3}M$ L-threonine	4	200	65,666 ± 4843
		4	500	36,368 ± 6319
		4	1000	18,769 ± 852
		4	2000	10,042 ± 1102
	$2 \times 10^{-3}M$ L-threonine	4	200	73,450 ± 5576
		4	500	45,300 ± 4529
		4	1000	27,937 ± 1971
		4	2000	16,875 ± 1639
	None	4	200	93,725 ± 7925
		4	500	64,550 ± 5794
		4	1000	40,920 ± 3415
		4	2000	28,300 ± 3706
L-valine-C14	$5 \times 10^{-3}M$ L-alanine	4	200	53,825 ± 3594
		4	500	31,070 ± 2400
		4	1000	22,400 ± 842
		4	2000	10,100 ± 597
	None	4	200	91,550 ± 4132
		4	500	75,902 ± 2926
		4	1000	41,850 ± 4903
		4	2000	28,475 ± 2685

penetration of L-serine demonstrated that valine competitively inhibits the entry of this amino acid (Table II).

Previous studies showed that at a concentration ratio of 2:1, glutamic acid did not inhibit the entry of L-valine. Therefore, it was surprising to find that aspartic acid is an effective inhibitor of L-valine entry. With L-valine at a concentration of 2×10^{-3} *M*, aspartic acid or proline at a concentration of 5×10^{-3} *M*, inhibited L-valine entry an average of 33 and 45%, respectively, in five experiments. A shortage of experimental material prevented a determination of whether or not aspartic acid is a competitive inhibitor.

L-lysine-C14 penetrates *Calliobothrium* at a very low rate. A series of experiments were performed to determine whether L-lysine or L-valine affect the entry of the other. L-lysine entry was not significantly affected by L-valine and L-valine entry was not affected by L-lysine (Table II). The apparent stimulation of L-valine entry by L-lysine at a lysine/valine ratio of 2, previously reported (Read, Simmons and Rothman, 1960), is not considered to be significant in view of the results obtained when this broader range of concentration ratios was examined.

When *Calliobothrium* was incubated for two minutes in 5×10^{-3} *M* L-valine-C14 and subsequently incubated for additional periods in salt medium without

TABLE II

Effects of certain amino acids on entry of L-serine, L-valine, and L-lysine. Data presented as in Table I

Amino acid	Inhibitor	N	1/S	V
L-serine-C14	$5 \times 10^{-3}M$ L-valine	4	200	45,600 ± 6111
		4	500	29,500 ± 8201
		4	1000	19,100 ± 1124
		4	2000	11,460 ± 823
	None	4	200	57,433 ± 2441
		4	500	42,150 ± 3300
		4	1000	29,100 ± 629
		4	2000	20,200 ± 4540
L-valine-C14	$5 \times 10^{-3}M$ L-lysine	4	200	105,975 ± 13,192
		4	500	73,050 ± 2563
		4	1000	47,300 ± 3379
		4	2000	32,750 ± 2958
	$2 \times 10^{-3}M$ L-lysine	4	200	97,025 ± 12,604
		4	500	72,025 ± 3620
		4	1000	46,250 ± 2418
		4	2000	31,433 ± 2240
	None	4	200	103,775 ± 14,202
		4	500	61,300 ± 963
		4	1000	47,300 ± 2588
		4	2000	34,050 ± 2952
L-lysine-C14	$5 \times 10^{-3}M$ L-valine	4	200	14,970 ± 1207
		4	500	7552 ± 1965
		4	1000	6247 ± 1406
		4	2000	5410 ± 441
	$2 \times 10^{-3}M$ L-valine	4	200	15,115 ± 3924
		4	500	7997 ± 1534
		4	1000	6713 ± 1408
		4	2000	5290 ± 805
	None	4	200	15,358 ± 1665
		4	500	7930 ± 1262
		4	1000	6232 ± 1836
		4	2000	5142 ± 909

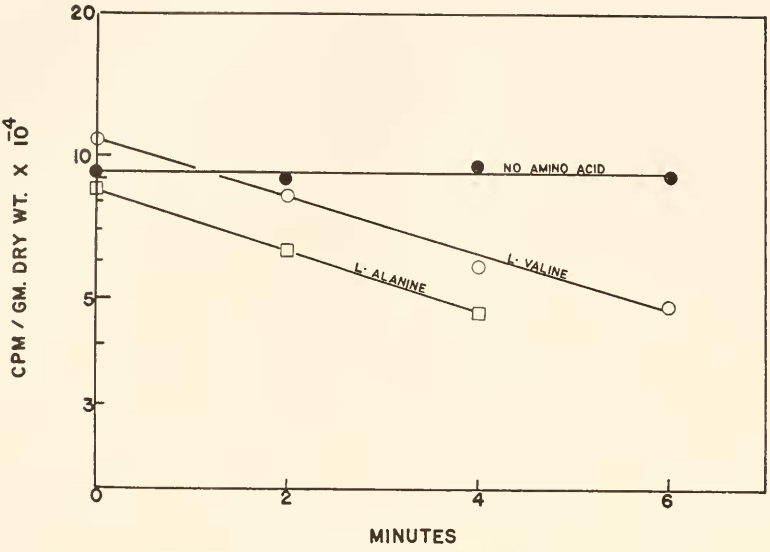

FIGURE 1. Loss of L-valine-C14 from *Calliobothrium* in the presence and absence of unlabeled amino acid in the suspending medium.

amino acid, "leakage" of L-valine from the tissues did not occur. However, when the worm, containing labeled amino acid, was placed in salt medium containing 5×10^{-3} *M* unlabeled L-valine, a decrease in the C14-labeled valine content of the worm was observed. This was analyzed by varying the time of incubation of labeled worms in medium containing unlabeled amino acid. The loss of labeled amino acid from the tissues followed a decay curve. A similar loss of L-valine-C14 from the worm occurred when the external medium contained unlabeled L-alanine (Fig. 1). In 6 experiments, incubation of worms in 5×10^{-3} *M* L-valine for 60 minutes had no effect on the entry rate of labeled valine in a subsequent two-minute period.

During the present study attempts were made to determine whether L-valine is concentrated against an electrochemical gradient. Worms were incubated for 40 minutes in L-valine-C14. The medium and the tissues were analyzed for free valine. The concentrations of valine inside and outside the worms are shown in

TABLE III

The accumulation of L-valine by Calliobothrium *against a concentration gradient. Incubation in* 9×10^{-3}*M L-valine for 40 minutes at 10° C.*

Sample	Final concentration in medium μM valine/ml.	Final concentration in worms μM valine/g. water	Worm/Medium
1	6.5	16.9	2.6
2	5.5	23.7	4.3
3	6.3	19.2	3.0
4	7.2	21.3	2.9

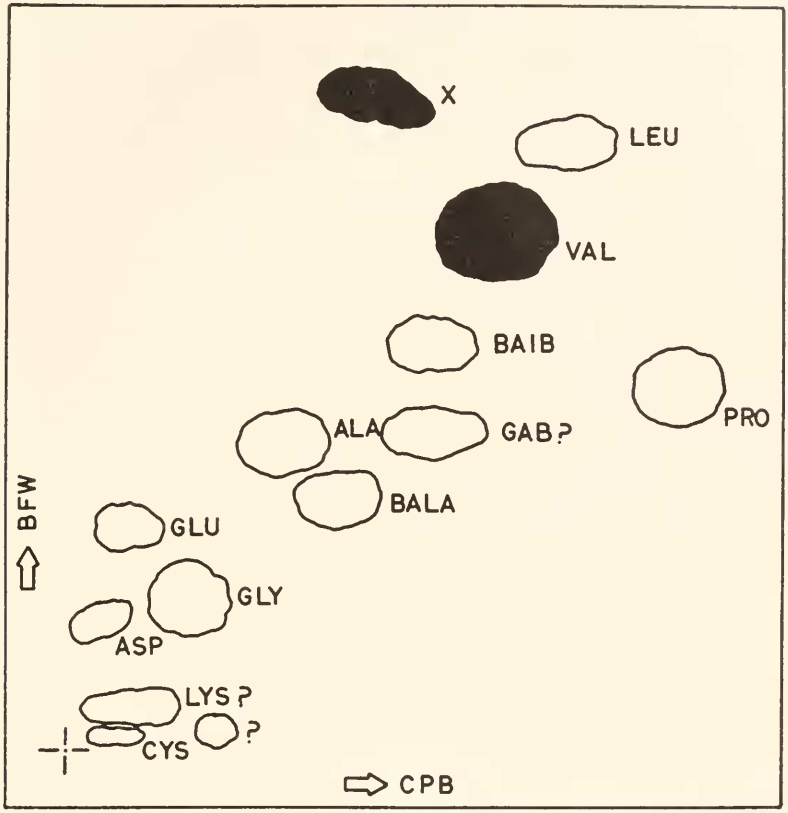

FIGURE 2. Chromatogram of free amino acids extracted from *Calliobothrium* after 40 minutes' incubation in presence of L-valine-C14. Solvent systems were sec-butyl alcohol, formic acid, water (75 : 15 : 10) = BFW and meta-cresol, phenol, borate buffer, ph 8.3 (60 : 30 : 15) = CPB. Black areas show presence of radioactivity, X being a non-ninhydrin-positive, unidentified metabolite.

Table III. It is apparent that valine is concentrated against a gradient. Radioautographs prepared from the chromatograms of tissue extract from the 40-minute incubations revealed that some L-valine is metabolized in this period. However, L-valine was the only radioactive ninhydrin-positive compound on the chromatograms. A second radioactive spot was present but did not react with ninhydrin. A representative chromatogram is shown in Figure 2. It was not feasible to characterize the unknown metabolite further. On the other hand, radioautographs prepared from 2-dimensional chromatograms of worm extracts from 2-minute incubations in L-valine-C14 revealed a single radioactive spot which proved to be identical with valine by the "fingerprint" method.

The amino acid entry systems of the host gut

Agar *et al.* (1956) differentiated and studied (1) the absorption of amino acids by the rat intestine *in vivo;* (2) the transfer of amino acids from inner to

TABLE IV

Uptake of L-valine-C14 by spiral valve tissue of Mustelus *spiral valve. Incubation in* 5×10^{-3} *M L-valine for 2 minutes at 10° C. Values are counts per minute per gram alcohol-extracted dry weight*

Spiral No. 2	Spiral No. 6
80,300	79,300
85,900	89,600
93,500	65,100
74,700	86,500
79,000	88,900
87,000	80,600
88,800	90,000
82,000	
Mean 83,900	82,857

outer fluids using loops of rat intestine; and (3) the uptake of amino acids by intestinal tissue. In short experiments, the kinetics of the latter two showed rather good agreement, although, as might be expected, a lag was observed in transfer experiments. Since the removal of amino acids from the lumen of the gut by tapeworms and by the host mucosa, rather than transport of amino acids in the

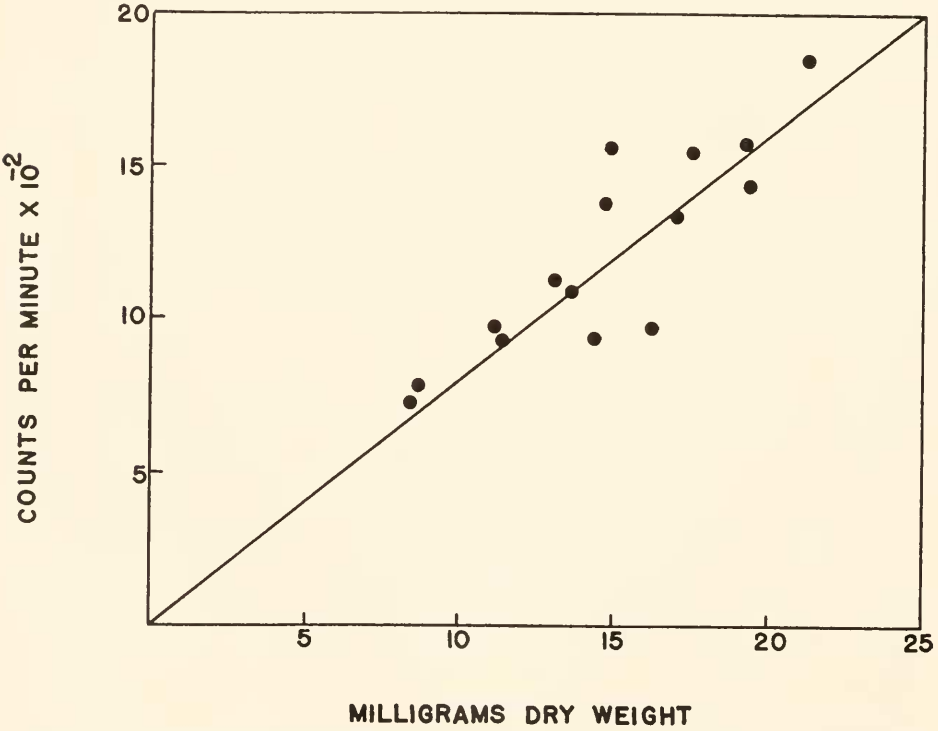

FIGURE 3. The quantity of labeled valine taken up by various amounts of spiral valve tissue of *Mustelus*. Tissue weight is alcohol-extracted dry weight.

extra-intestinal host tissues, is the primary biological aspect to be considered here, experiments to study the uptake of amino acids by dogfish intestinal tissues were carried out. The preparations used were pieces from the lamina of the spiral valve cut approximately 1 cm. square. As many as 30 such pieces can be obtained from the lamina of a single spiral turn in the intestine of a sexually mature dogfish. Most of the experiments to be described were carried out with tissues from the fourth spiral posterior to the pyloric valve, although, as will be shown below, preparations from other spirals would probably yield quite comparable data. *Calliobothrium* is found predominantly in the region of the fourth and fifth spirals. The methods of handling the dogfish tissue were de-

TABLE V

Reciprocal inhibition of L-serine and L-valine entry and L-lysine entry into spiral valve tissue of Mustelus canis. *Data presented as in Table I. For experimental details, see text*

Amino acid	Inhibitor	No.	1/S	V
L-serine-C14	None	4	200	42,000 ± 3725
		4	500	28,700 ± 4340
		4	1000	14,900 ± 1859
		4	2000	7695 ± 1236
	$5 \times 10^{-3}M$ L-valine	4	500	10,666 ± 1231
		4	1000	6056 ± 586
		4	2000	2966 ± 561
L-valine-C14	None	4	200	68,980 ± 8271
		4	500	35,630 ± 6643
		4	1000	21,996 ± 5036
		4	2000	13,286 ± 1306
	$5 \times 10^{-3}M$ L-serine	4	200	32,666 ± 2714
		4	1000	15,860 ± 2286
		4	2000	9760 ± 1699
L-lysine-C14	None	4	200	27,293 ± 646
		4	500	17,551 ± 3409
		4	1000	10,800 ± 1184
		4	2000	6575 ± 1197

scribed earlier. It was reasoned that if preparations from different parts of single or separate spiral lamina of the intestine showed consistency in rate of amino acid uptake, with respect to weight of tissue, replicate samples from a single fish could be used in amino acid uptake studies. Initially, therefore, a determination of amino acid uptake was made with tissue samples removed from the second and sixth spirals. Data obtained with tissues from a single fish are presented in Table IV and show remarkably that there is no significant difference in the entry of L-valine into tissues from these two regions of the intestine nor into tissues from different parts of the same lamina. Further, the amount taken up is proportional to the dry weight of tissue used (Fig. 3). These findings showed that multiple sampling for kinetic studies is feasible with these preparations.

TABLE VI

Effects of other amino acids on entry of L-valine into spiral valve tissue. V = counts/min./gram alcohol-extracted dry tissue. N = Number of experiments

Inhibitor	N	V
Glycine	4	98,771 ± 6129
L-leucine	4	57,692 ± 3406
L-isoleucine	4	44,326 ± 1267
L-methionine	4	40,253 ± 3294
L-threonine	4	71,655 ± 4029
L-lysine	4	79,363 ± 2326
None	4	91,799 ± 1079

When the concentration of L-valine was varied, it was found that the entry of the amino acid into mucosal tissues follows an adsorption isotherm, and the apparent Michaelis constant for valine entry is about 5×10^{-3}. The addition of unlabeled L-serine at a concentration of 5×10^{-3} produced an inhibition of L-valine entry which was competitive in nature (Table V.) Conversely, L-valine competitively inhibits L-serine entry (Table V) which has an apparent Michaelis constant of about 4.5×10^{-3}.

A number of other amino acids were tested as inhibitors of L-valine entry. Data obtained are summarized in Table VI. It was found that "preloading" the mucosa, by incubating for 40 minutes in non-radioactive L-valine, produced no effect on the subsequent entry rate of L-valine-C14. In balanced salt solution without added non-radioactive amino acid, L-valine does not leak out of the intestinal tissue to a significant extent.

TABLE VII

Relative concentrations of free amino acids in the fluid contents of the spiral valve of Mustelus canis. *All values are related to a valine concentration of 1.00. mg. N = Mg. of alcohol-soluble nitrogen in the sample*

Amino acid	Dogfish No.												$\bar{X} \pm$ S.E.
	1	2	3	4	5	6	7	8	9	10	11	12	
Leucine	1.43	1.46	1.20	1.53	1.43	1.67	1.44	1.15	1.18	1.38	1.61	1.41	1.41 ± 0.047
Phenylalanine	0.77	0.77	0.53	0.71	0.47	0.58	0.62	0.54	0.84	0.57	0.75	0.59	0.64 ± 0.034
Valine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tyrosine	0.76	0.91	0.55	0.94	0.43	0.65	0.82	0.63	0.90	0.50	0.75	0.70	0.71 ± 0.049
Alanine	1.35	0.78	0.87	1.94	1.50	1.42	1.65	1.21	1.13	1.25	1.66	1.51	1.36 ± 0.096
Threonine	0.58	0.42	0.35	0.65	0.78	0.49	0.88	0.75	0.83	0.72	1.07	0.84	0.70 ± 0.060
Glutamic	1.98	1.34	1.61	4.24	2.66	3.63	7.65	2.70	2.42	1.99	2.87	2.92	3.00 ± 0.49
Glycine & serine	1.61	1.18	1.72	5.41	2.90	3.12	4.61	2.41	6.26	4.13	3.44	3.14	3.33 ± 0.45
Aspartic	0.95	0.67	1.11	2.47	1.52	1.43	3.06	1.15	2.60	3.50	1.38	3.46	1.94 ± 0.29
Lysine	1.37	1.00	1.70	5.77	7.05	2.27	1.85	1.78	2.04	3.32	6.97	2.44	3.13 ± 0.63
Histidine	0.51	0.48	0.73	—	—	0.77	1.01	0.69	1.09	0.33	1.61	0.51	0.64 ± 0.095
Arginine	0.46	0.57	0.53	—	—	0.39	0.82	0.60	0.86	0.35	0.59	0.33	0.46 ± 0.078
Cysteine	—	—	—	—	0.84	0.70	—	0.52	—	—	—	0.21	0.19 ± 0.090
Beta aminoiso-butyric	—	—	—	—	0.18	0.19	—	0.34	—	0.26	—	—	0.081 ± 0.037
Beta alanine	—	—	—	—	—	0.24	1.94	2.21	0.99	0.55	1.44	—	0.62 ± 0.23
mg. N	4.70	1.43	2.27	4.20	7.40	3.35	4.84	3.28	2.60	5.80	3.00	9.40	

Using dogfish gut preparations, attempts were made to show the inhibition of L-histidine uptake by other amino acids in experiments of 30-minute duration. L-histidine was taken up by the tissues and, during the experimental period, molar ratios of histidine in the tissue water/histidine in the external fluid of more than 2 developed. Initial concentration of histidine in external fluid was 1 mM. Addition of L-alanine, L-proline, L-valine, L-serine, or L-aspartic acid at a concentration of 2.5 mM. did not affect L-histidine uptake significantly. Histidine was removed from the medium at the rate of 20 to 40 micromoles per gram dry weight per 30 minutes.

Free amino acids of the intestinal lumen

Samples of the fluid contents of the spiral intestine were collected from a number of dogfish. There was great variation in the nutritional state of these animals. Some were freshly captured and some had been held in captivity for as long as 8 days without food. The samples were taken with a calibrated pipette from the middle portion of the spiral valve of living fish. The measured volume was immediately added to 10 volumes of 70% ethyl alcohol, mixed, and allowed to settle for several days. Free amino acids in the supernatant liquid were quantitatively determined. The analyses are summarized in Table VII. It is evident that there is great variation in the absolute quantity of alcohol-soluble nitrogen and of single amino acid components. However, study of the data shows that, for the most part, the molar ratios of one amino acid to another are strikingly constant. The dicarboxylic acids, aspartic and glutamic acid, show considerable variation, but it may be seen that the ratio aspartic/glutamic is relatively stable. The lysine/valine ratio also showed considerable variation. This may be associated with the relatively low rate at which lysine penetrates the mucosa. The methods used did not allow a clear separation of glycine and serine and these two amino acids were estimated together. However, it was roughly estimated that serine made up about 75 per cent of this value.

Discussion

The demonstration that certain amino acids, which inhibit the entry of L-valine into *Calliobothrium*, do so competitively, as previously shown with L-leucine (Read, Simmons and Rothman, 1960) suggests that this may also be the case with other inhibitory amino acids. Limitations of time and material have not allowed a complete analysis of the inhibitions of valine entry produced by cysteine, methionine, glycine, or proline.

Furthermore, some of the amino acids which do not inhibit the entry of L-valine or L-leucine at a concentration ratio of 2:1 may very well inhibit at higher concentration ratios. D-valine was found to inhibit L-valine entry at a very high D/L ratio but had no significant effect when D-valine/L-valine was 2 (Read, Simmons and Rothman, 1960). Of great interest is the failure of mutual competition between L-lysine and L-valine over a wide range of concentration ratios. Since L-lysine entry appears to follow adsorption kinetics, the lack of interaction with L-valine entry suggests that the two compounds enter at different sites.

The inhibition by other amino acids of L-valine and L-serine entry into the

intestinal tissue of the dogfish, shown to be mutually competitive in the case of L-serine and L-valine, is consistent with the observations of others who have reported inhibition of intestinal absorption of single amino acids by other amino acid species in warm-blooded vertebrates (Wiseman, 1955, 1956; Agar *et al.*, 1956).

A difference, however, is observed in the case of L-histidine uptake by dogfish and rat intestinal tissues. Agar *et al.* (1956) found that L-histidine uptake was markedly inhibited by addition of equimolar concentrations of a number of single amino acids. Inhibition was not observed with dogfish tissues when several amino acids were singly added at twice the histidine concentration. Christensen and his co-workers (reviewed by Christensen, 1959) have described reciprocal inhibitions of amino acid uptake with Ehrlich ascites tumor cells and such relationships between amino acids are known to occur with *Neurospora* (Mathieson and Catchside, 1955)

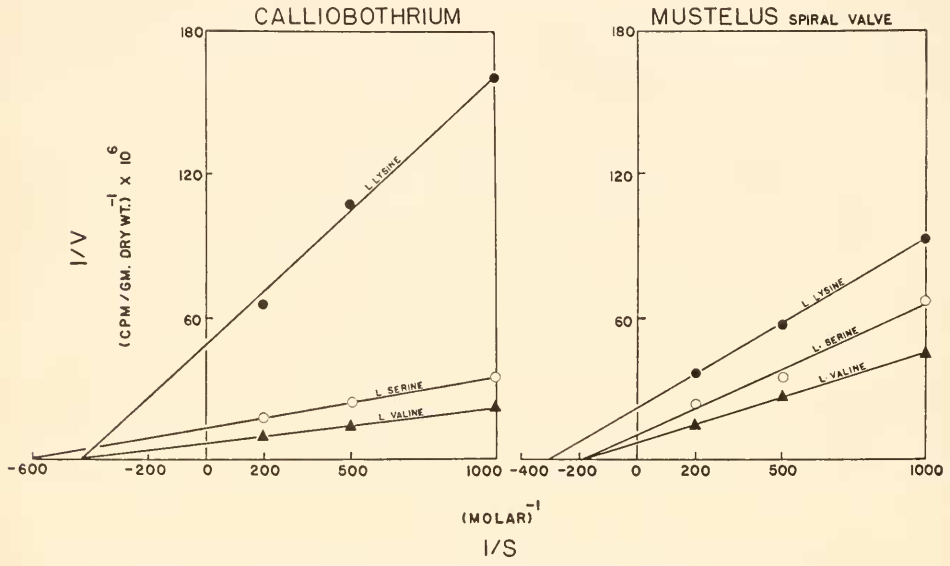

FIGURE 4. A kinetic comparison of the uptake of three amino acids by *Calliobothrium* and *Mustelus* spiral valve tissue.

and certain bacteria (Cohen and Monod, 1957). It appears to be a very general phenomenon, perhaps as general as the distribution of special membrane mechanisms for amino acid entry. It seems unfortunate that some of the bacterial physiologists have chosen to refer to such entry systems as "permeases," a term implying that there is an entry enzyme. It is to be hoped that a term with such specific connotations is not widely adopted until considerably more understanding of mechanism is attained.

There are definite differences in the amino acid entry systems of *Calliobothrium* and the dogfish intestinal tissue. In Figure 4 the worm and host tissue are compared with regard to their affinities for three amino acids.

Several years ago the senior author reviewed the literature on the physiology of the small intestine with special reference to its peculiarities as a habitat (Read,

1950). It was concluded that there is a flow of organic compounds, with the notable exception of carbohydrates, from the tissues into the gut lumen and that much of this material is resorbed in areas of the gut distal to the point of secretion. It seemed obvious to infer that many of these materials are available to lumen-dwelling parasites. The present data on the free amino acids in the gut lumen of dogfish in highly variable states of nutrition, and the great stability of molar ratios of these amino acids lend weight to the above concept. As a part of a study of mammalian nutrition, Nasset and his colleagues (1955) demonstrated that molar ratios of free amino acids, one to another, in the small intestine of the dog are astonishingly constant and independent of the composition of protein ingested. The concentration ratios were essentially unchanged in dogs receiving *no* protein by mouth. This has broad implications in considering intestinal parasitism. It reinforces the senior author's argument that in many chemical characteristics, the small intestine represents a relatively stable environment in a particular host. More specifically, it invites inquiry as to what effects constancy of the relative amounts of different amino acids might have on a particular intestinal parasite. It seems plain that if the environment offers a mixture of amino acids or other compounds of nutritional significance, and if these compounds compete with or otherwise affect the entry of one another into the tissues of the parasite, the ratios of amino acids in the mixture will be extremely important in determining whether the nutritional requirements of the parasite can be maintained in a balanced state. The concept emerges that the ratios of nutrients may be *critical* in determining whether or not a given mixture of amino acids will represent a satisfactory food for a parasitic organism such as a tapeworm. Thus, ratios of nutrient concentration may be critical limiting parameters at the *interface* between host and parasite. Further, the ratios of rates of entry of amino acids may be manifestations of an important regulatory system governing the make-up of the amino acid pool in a given worm.

As a homeostatic mechanism of importance in the physiology of the vertebrate, the competitions between amino acids would seem to represent part of a mechanism for regulating the composition of the amino acid mixture entering the portal system and hence the liver. Nasset (1957) has presented evidence that the relative concentrations of amino acids in the small gut are maintained by the secretion of endogenous nitrogenous material which is mixed with the ingesta. It would seem that the regulation of the composition of the amino acid pool for protein synthesis in the vertebrate begins at the mucosa.

While it would appear that the host and the parasite are competing with each other as whole organisms and, from this standpoint, the competition of worm and host should be considered in terms of total absorption by worm and intestine, we may consider highly localized competition in terms of the entry systems for a particular amino acid. If other amino acids affect entry of this amino acid into the mucosa and the worm to differing extents, it is apparent that the concentration ratios of amino acids may undergo alteration in the immediate vicinity of the worm-mucosa system. If the rates involved do not undergo marked change, a new set of concentration ratios should be established. If this is indeed true, it may have wider implications in considering parasitisms in which the most obvious effects on the host are general unthriftiness or ill defined interferences with nutrition. It has not been feasible to study this experimentally in the dogfish-cestode system

but it may be practical with other host-parasite combinations more amenable to laboratory control.

It becomes increasingly apparent that the gut should not be considered a space outside the vertebrate body. The rapid changes in the properties of intestinal mucus indicating hydrolysis of components (Hartiala and Grossman, 1952) suggest that secretion, hydrolysis, and resorption must occur constantly. Lumen parasites are in a position to remove from this exocrine-enteric circulation compounds of nutritional value. If the data on rate of entry of individual amino acids into *Calliobothrium* and host intestinal tissue are calculated on the basis of water content, making the assumption that the amino acids are in solution in this water, the tapeworm takes up the amino acids studied at a much higher rate than host intestinal tissue. However, the data on competitions indicate that rates of absorption for single amino acids are not directly applicable to complex mixtures. Study of entry of single components from complex mixtures is obviously required.

Summary

1. The entry of C14-L-valine into the tapeworm, *Calliobothrium verticillatum*, is competitively inhibited by L-serine, L-threonine, and L-alanine. Conversely, L-valine competitively inhibits the entry of C14-L-serine.

2. The entry of C14-L-valine is not significantly affected by L-lysine and, conversely, L-lysine entry is not affected by L-valine.

3. L-valine is concentrated against a gradient by *Calliobothrium* in experiments of 40-minute duration.

4. The entry of C14-L-valine into mucosal tissues of the dogfish host, *Mustelus canis*, is competitively inhibited by L-serine and, conversely, C14-L-serine entry is competitively inhibited by L-valine. L-leucine, L-isoleucine, L-methionine, L-threonine, and L-lysine also inhibit C14-L-valine entry but it has not been shown that inhibition is competitive.

5. In experiments of 30-minute duration, L-histidine uptake by dogfish mucosa was not affected by L-alanine, L-proline, L-valine, L-serine, or L-aspartic acid at the concentrations tested.

6. Quantitative analyses of free amino acids of the dogfish intestinal lumen showed variability in the absolute concentrations but great stability in the relative concentrations.

7. The data are discussed in terms of differences in amino acid entry systems of host and parasite, the significance of stability of amino acid ratios in the nutrition of host and parasite, and the necessity for evaluating host-parasite competitions in terms of entry of single components from complex mixtures.

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